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NCBI Coffee Break

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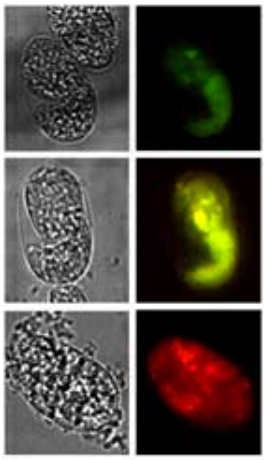
Coffee Break archives

nature REVIEWS Story contributed by Raluca Gagescu , Nature Reviews Molecular Cell Biology

Fluorescent timer



Change in fluorescence. E5 changes its fluorescence from green to red over time.

Click on the figure for more information.

There are many ways to monitor the onset of gene expression, but so far it has been impossible to detect its down-regulation. This problem might have been solved now, as Terskikh and colleagues report in *Science* a simple method to follow promoter activity.

Last year, a red fluorescent protein (drFP583) was identified in tropical corals, further increasing the wide spectrum of possibilities to light up cells in different colors. Not satisfied with just one color, Terskikh and colleagues introduced random mutations into drFP583, and found one mutant (called E5) that changes its fluorescence from green to red in a time-dependent manner. As E5 switches from green to red fluorescence over time, it can be used as a timer for gene expression. During the first hours of activity of a promoter, green fluorescence is predominant, whereas sustained activity of the promoter leads to a mixture of green and red fluorescence. A few hours after the promoter is turned off, only red fluorescence remains.

Terskikh and colleagues verified these predictions in three experimental systems. First they monitored up- and down-regulation of E5 expression in Tet-on and Tet-off mammalian expression systems. Then they followed the activity of a heat-shock promoter during heat-induced stress of *Caenorhabditis elegans.* Last, they traced the expression of a homeobox gene involved in the patterning of anterior structures in *Xenopus laevis.* In all cases, green fluorescence correctly indicated the onset of gene expression and was replaced with red fluorescence when expression ceased.

So after decades of blue-stained embryos, we'll now have to get used to seeing gene expression in green and red.

Comments?

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A fluorescent timer

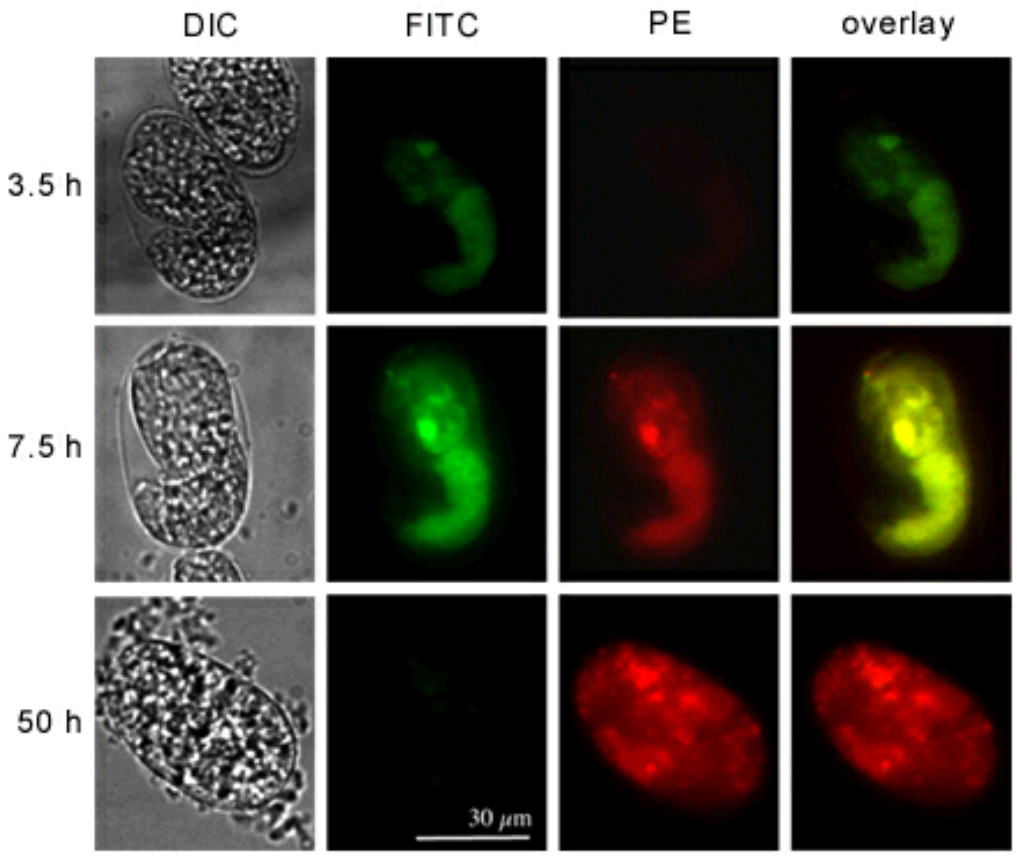

Figure 1. The change in fluorescence of E5 over time in *C. elegans*. The *E5* mutant was placed under the control of a heat shock promoter and injected into *C. elegans* embryos. Green fluoresence was detected 2 hours into the recovery phase following a standard heat shock treatment (1 hour incubation at 33¡C). The embryos were documented under bright field (DIC), with a FITC filter, with a PE filter, and with an overlay at 3.5, 7.5, and 50 hours following heat shock. Yellow fluorescence, as seen in the overlay column at 7.5 hours, indicates a combination of green and red fluorescence.

Figure generously provided by Dr. Alexey Terskikh, Stanford University.

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This tutorial will demonstrate how to search for neighbors of a newly determined structure. Before searching the MMDB database with PDB information, make sure that the structure is not already present in the database (see the Coffee Break article dated 6 Nov. 2000 in the archives for a tutorial on how to do this).

To begin a search for structural neighbors, click on the link marked by the red arrow below. (Note: selecting other links will take you out of this tutorial.)

VAST Search

VAST Search is NCBI's structure-structure similarity search service. It compares 3D coordinates of a newly determined protein structure to those in the MMDB/PDB database. VAST Search computes a list of structure neighbors that you may browse interactively, viewing superpositions and alignments by molecular graphics.

- To search for neighbors of a newly determined structure:
  - If you used X-PLOR to generate your PDB file <CLICK HERE>.
  - If your have a CNS deposit file <CLICK HERE>.
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- To view results of an earler search go <CLICK HERE>.
- To view VAST neighbors of a structure already in MMDB/PDB <CLICK HERE>.

Tutorial Coffee Break

Structural alignments may be searched for on the basis of user-defined PDB files. Initiation of a VAST Search requires that the fields below be completed. (The necessary information for this tutorial has already been supplied.) To continue, click on the "Submit Request" button marked by the red arrow at the bottom of the page.

Click here to read all the instructions.

Contact information just in case the request fails:

Name: John Doe Email: j_doe@....com

Please specify a password so that results can be viewed confidentially:

Password: password

Write it down! It is needed to view results online. Passwords must be at least six characters.

Please read about confidentiality of VAST Search.

A pdb file is required

Please upload the **pdb** file here: Ds_Red3D.pdb Browse

Note: Please remove all records except ATOM records when possible.

Does this structure contain multiple chains?

○ **Yes** ◉ **No**

What search set of the Protein Data Bank do you want to do your VAST search against?

◉ **Non-redundant Subset of PDB** ○ **All of PDB**

Submit Request ← Reset Request

Items may be selected for 3D alignment by checking the box that is to the left of the PDB Id. 'Green Fluorescent Protein' has been marked for selection (see below). Click on the 'View/Save Alignments' button above to see the selected structure aligned with DsRed.

Structures similar to VAST Search VS135...

View / Save Alignments NEW *Get Cn3D 2.5!*

Options:
- (•) Launch Viewer
- ( ) See File
- ( ) Save File

Viewer:
- (•) Cn3D v2.5 (asn.1)
- ( ) Mage (Kinemage)
- ( ) (PDB)

Complexity:
- (•) Aligned Chains only
- ( ) All Chains
- (•) Alpha Carbons only
- ( ) All Atoms

Structure neighbor 1 out of 1 displayed. Page 1 of 1.

	PDB C D	RMSD	NRES	%Id	Description
☑	1GFL A	1.1	222	21.2	Structure Of Green Fluorescent Protein

Display / Sort Hits page number: 1 Hits to display per page: 20 choose between 20-100 neighbors per page.

Display Subset:
- (•) Non-redundant; BLAST p-value 10e-7
- ( ) Non-redundant; BLAST p-value 10e-40
- ( ) Non-redundant; BLAST p-value 10e-80
- ( ) Non-identical sequences
- ( ) All of MMDB

Sorted by:
- (•) VAST Score
- ( ) VAST P-value
- ( ) Rmsd
- ( ) Aligned residues
- ( ) Identities

Column Format:
- (•) RMSD, NRES, %Id
- ( ) All values

Analysis Coffee Break

A fluorescent timer

Structural alignment of drFP583 structure with green fluorescent protein using the Cn3D viewer

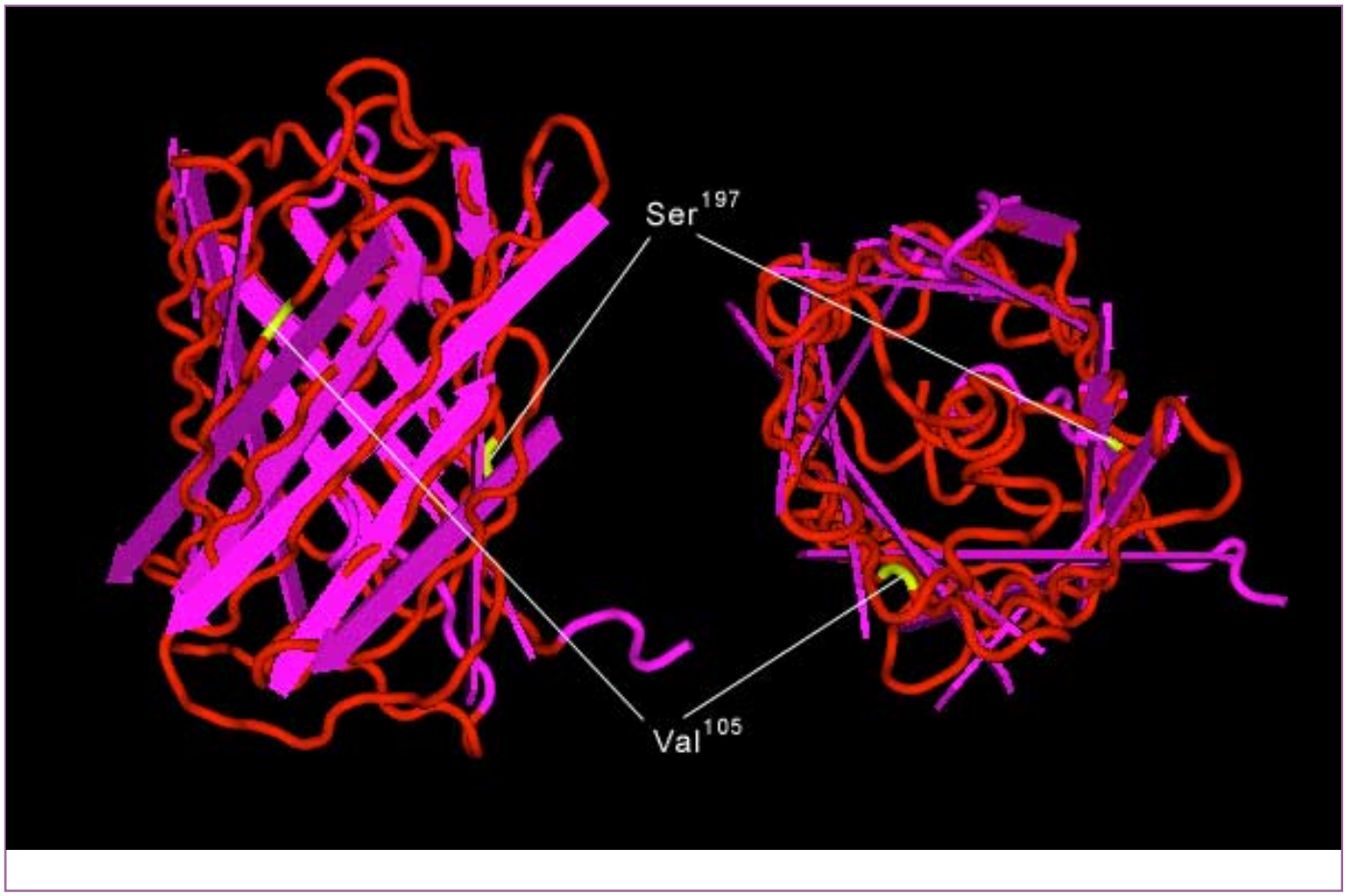

Figure 1

Figure 1 depicts the 3-dimensional ribbon structure of the red fluorescent protein (DsRed), drFP583. In the full crystal structure, four monomers of DsRed associate to form a stable tetramer [1]. Each monomer of DsRed is comprised of an 11-strand β-can, that surrounds a central α-helix chromophore. The structure in Figure 1 depicts a monomer of DsRed isolated from the tetrameric crystal structure. To download this structure alignment in Cn3D format, click on the Figure 1 image or click here.

Green fluorescent protein (GFP) was originally found in the luminescent jelly fish *Aequorea victoria.* This important protein aids in scientific research by serving as a convenient marker that signals gene activity as well as functioning as a sensitive label [2, 3]. The search for other proteins that exhibit fluorescent properties led to the discovery of GFP-like fluorescent proteins in coral reefs [4]. Experimental mutation of the red coral protein drFP583 resulted in the creation of E5, a protein whose fluorescence changes over time [5]. It was determined that two amino acid substitutions exist between drFP583 and E5. Valine 105 is changed to alanine and Serine 197 is changed to threonine in the E5 mutant (these amino acid positions are highlighted yellow in Figure 1, above) [5].

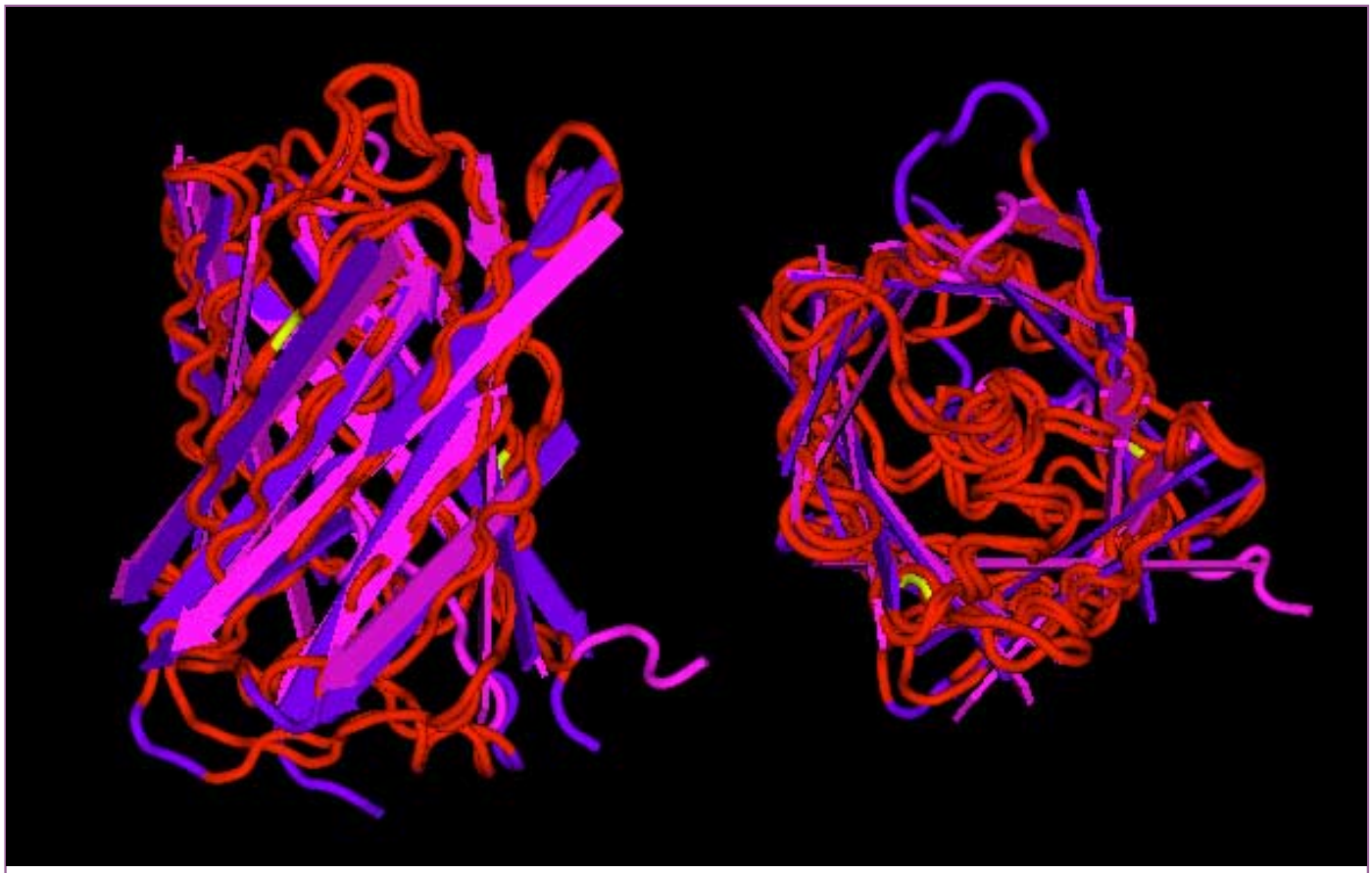

Figure 2

The red coral protein drFP583 is shown aligned with GFP in Figure 2. Pink chains correspond to DsRed, purple chains correspond to GFP, and red chains are indicative of high structure similarity between DsRed and GFP. To download this structure alignment in Cn3D format (note: same file as Figure 1), click on the Figure 2 image or click here.

The PDB coordinates of DsRed (PDB Id=1ggx) was used to search the MMDB/PDB database using the VAST program [6] with standard parameters. Green fluorescent protein, included in the alignment with DsRed, was selected from the output of the first iteration. The multiple sequence alignment was constructed using Cn3D 3.0 [7].

[1] Wall MA, Socolich M, and R Ranganathan (2000) The structural basis for red fluorescence in the tetrameric GFP homolog DsRed. *Nat Struct Biol* 7, 1133-8.

[2] Kendall JM and MN Badminton (1998) *Aequorea victoria* bioluminescence moves into an exciting new era. *Trends Biotechnol* 16, 216-24.

[3] Tsien RY. (1998) The green fluorescent protein. *Annu Rev Biochem* 67, 509-44.

[4] Matz MV, et al. (1999) Fluorescent proteins from nonbioluminescent Anthozoa species. *Nat Biotechnol* 17, 969-73.

[5] Terskikh A, et al. (2000) "Fluorescent Timer": protein that changes color with time. *Science* 290, 1585-8.

[6] Madej T, et al. (1995) Threading a database of protein cores. *Proteins* 23, 356-369.

[7] Wang Y, et al. (2000) Cn3D: sequence and structure views for Entrez. *Trends Biochem Sci* 25, 300-302.